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Role of galectin-8 as a modulator of cell adhesion and cell growth

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Galectin-8 belongs to the family of tandem-repeat type galectins. It consists as several isoforms, each made of two domains of ∼140 amino-acids, both having a carbohydrate recognition domain (CRD). These domains are joined by a 'link peptide' of variable length. The human galectin-8 gene covers 33 kbp of genomic DNA. It is localized on chromosome 1 (1q42.11) and contains 11 exons. The gene produces by alternative splicing 14 different transcripts, altogether encoding 6 proteins. Galectin-8, like other galectins, is a secreted protein. Upon secretion galectin-8 acts as a physiological modulator of cell adhesion. When immobilized, it functions as a matrix protein equipotent to fibronectin in promoting cell adhesion by ligation and clustering of a selective subset of cell surface integrin receptors. Complex formation between galectin-8 and integrins involves sugar-protein interactions and triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK and paxillin. In contrast, when present in excess as a soluble ligand, galectin-8 (like fibronectin) forms a complex with integrins that negatively regulates cell adhesion. Such a mechanism allows local signals emitted by secreted galectin-8 to specify territories available for cell adhesion and migration. Due to its dual effects on the adhesive properties of cells and its association with fibronectin, galectin-8 might be considered as a novel type of a matricellular protein. Galectin-8 levels of expression positively correlate with certain human neoplasms, prostate cancer being the best example studied thus far. The overexpressed lectin might give these neoplasms some growth and metastasis related advantages due to its ability to modulate cell adhesion and cellular growth. Hence, galectin-8 may modulate cell-matrix interactions and regulate cellular functions in a variety of physiological and pathological conditions.
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Introduction

Galectin-8 [1], is a 34 kDa protein made of tandem-repeat carbohydrate recognition domains (CRDs), joined by a 'link' peptide. *In vitro* translation products of galectin-8 cDNA or bacterially-expressed recombinant galectin-8 (rGalectin-8) are biologically active, possess sugar-binding and express strong hemagglutination activity [1]. In many aspects, galectin-8 resembles other tandem-repeat type galectins; nonetheless, several features of galectin-8 single it out of the entire galectin family. These features are discussed in this review.

Protein structure of galectin-8

Galectin-8 was originally cloned from a rat liver cDNA library. The isolated clone contained an open reading frame that codes for 316 amino acids, which forms a protein of about 35 kDa. Structurally, galectin-8 belongs to the tandem-repeat type galectins. Other members of this family are a 32-kDa galectin from Caenorhabditis *elegans* [2] (CE-galectin), as well as galectin-4 [3]; -6 [4], -9 [5], and -12 [6]. All members of this group contain two CRDs connected by a link peptide (Figure 1). The N- and C-terminal domains share ∼35% homology, and both domains contain sequence motifs (*e.g.* HXNPR; WGXEE) that have been conserved among most carbohydrate recognition domains of galectins [7,8]. At the level of amino acids, galectin-8 shares ∼30–40% identity with other tandem-repeat type galectins, but no homology with any known protein is found in the region of the link peptide. Galectin-8 contains four potential N-linked glycosylation (Asn-X-Ser/Thr) sites. However, the fact that denatured and reduced hepatic galectin-8 has a similar mobility on SDS-PAGE as its bacterially-expressed recombinant counterpart (rGalectin-8) [1], suggests, though not proves, that native galectin-8 is neither heavily glycosylated, nor it is subjected to extensive post-translational modifications (*e.g.* phosphorylation).

The predicted secondary structure of galectin-8 reveals that its N- and C-terminal domains are structurally homologous,

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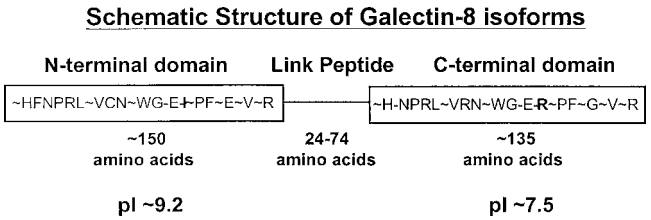


Figure 1. Galectin-8 encodes for a galectin with two homologous carbohydrate-binding regions. A schematic structure of galectin-8 is presented. Each box represents a putative carbohydrate-binding domain, linked by a 24–74 amino acid long peptide. Shown are selected invariant amino acids preserved in most galectins analyzed so far.

as expected from their primary structure. Both domains are predicted to form several β-sheets, a structural feature of other galectins [9,10]. Based upon the known crystal structure of galectin-1 homodimer [11], a model structure of galectin-8 was built. Galectin-8 presumably consists of continuous two 10- and 12-stranded anti parallel β-sheets that extend in a two-fold symmetric fashion across the interface of its N- and C-terminal domains. The carbohydrate binding sites are at the far-end of each domain, and no disulfide links are predicted (Figure 2).

Although galectin-8 contains two CRDs, potential differences in sugar-binding between the domains is predicted from a critical difference in their sequence [WGXEX**I** vs. WGXEX**R** at the N- and C-terminal CRDs of galectin-8, respectively (cf. Figure 1)]. The (bold) Arg residue has been implicated as playing an important role in the interactions between galectins and the glucose moiety of lactose [12]. Furthermore, site-directed mutagenesis studies [13] indicated that substitution of this conserved Arg for His, abolishes sugar binding. The presence of Ile90 (instead of an Arg) at the N-terminal CRD of galectin-8 suggests that galectin's CRD might accommodate non-basic amino acids at this site, which might result in alteration of its sugar-binding specificity. Hence, galectin-8 could function as a hetero-bifunctional crosslinking agent and therefore have a broadened range of interactive capacity. Cloning of several human homologues of galectin-8 [14,15], in which the WG-E-**I** motif is conserved, lends further support to this notion, and suggests that this unique architecture is presumably a common feature of all isoforms of galectin-8.

Genomic structure of galectin-8

Galectins containing tandemly repeated CRD domains, such as galectin-8, presumably arose during evolution by duplication and subsequent divergence of relevant exons from genes encoding prototype galectins. The ~35% amino acid similarity between the N-CRD and C-CRD of galectin-8 substantiate this notion [1]. The human galectin-8 gene covers 33 kbp of genomic DNA. It is localized on chromosome 1 (1q42.11) and contains 11 exons. Exons 2–11 contain the coding sequences. The gene produces by alternative splicing 14 different transcripts, altogether encoding 6 proteins. The mRNA of most transcripts has a short 5′-UTR, but the 3′UTR is among the 5% longest found to date, and as such it may serve a regulatory function. Of interest, the galectin-8 gene, is antisense over ~1 kbp, both at its 5′ and 3′ ends, to two separate gene, each encoding a protein of yet unknown function. Hence, the possibility that galectin-8 mRNAs may be present and stable only in the absence of matching mRNAs from genes on the other strand and *vice versa* should be considered.

Galectin-8 isoforms

The deduced amino acid sequences of rat and human galectin-8 reveal that the galectin-8 gene encodes at least six isoforms of the protein [15–17]. All six isoforms contain the N-terminal region of galectin-8, with its unique WGXEXI "signature motif". Two of the six isoforms are, in fact, prototype galectins because they lack the C-terminal domain of galectin-8, and contain only the N-terminal domain, fused to 'hinge' regions bearing different length insertions. The four isoforms that are of the tandem-repeat type, also differ in the size of their "hinge region" which varies in length from 24 to 74 amino acids. The presence of at least six isoforms of galectin-8 indicates that this lectin should be considered as a sub-family among the galectins. The variable length of the "hinge region" is likely to affect the repertoire of the glycoproteins which interact with the tandem-repeat type galectin-8, because the two CRDs spaced at different distances are likely to bind different spatially oriented carbohydrates. This may affect the function of galectin-8 and may account for the presence of its different isoforms.

Galectin-8 as a secreted protein

Galectin-8, like other galectins, is a secreted protein [18]. Accordingly, galectin-8 is found on the surface of several cell lines, and it can be released from the cell surface by trypsinization [18]. Hence, galectin-8, like other galectins, is expected to function, at least in part, extracellularly. Having no signal peptide, the mode of secretion of galectins is largely unknown. However, it has been previously shown that secreted galectins are concentrated in evaginations of the plasma membrane, which pinch off to form labile lectin-rich extracellular vesicles which may interact with cell surface proteins [10,19–21]. Atypical secretion is not a unique property of galectins, since other cytoplasmic proteins like thioredoxin [22], interleukin-1β [23] and basic FGF [24] lack a signal sequence, yet are externalized and function extracellularly.

In that respect, it is of interest to note that recombinant galectin-8 remains soluble, and maintains sugar-binding capacity even when extracted and purified in the absence of reducing agents [25]. These findings are compatible with the notion [26] that a reducing environment is not necessarily required to maintain sugar binding of galectins. This conclusion has somewhat broader implications and supports the notion that at least

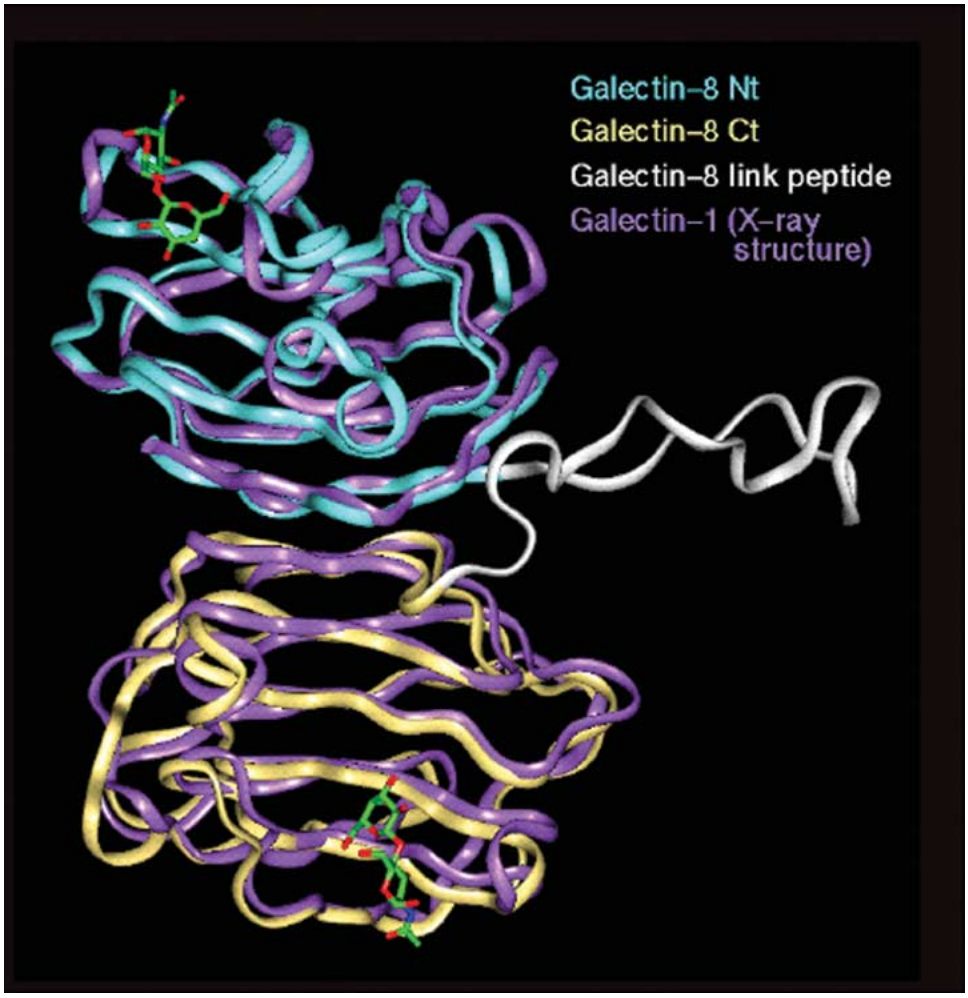


Figure 2. Predicted three-dimensional structure of Galectin-8. A model structure of galectin-8, based upon the known structure of galectin-1 [76] and galectin-2 [12] homodimers (~34% identity), was built using the Homology (Biosym, San Diego, CA) program. The models were mainly based upon the structure of galectin-1 except for two loops (one in each domain) which were more similar to the corresponding loops in galectin-2. The initial model of the hetero-dimer was energy minimized using the program Encad [77]. The Cα atoms were restricted to their initial positions so that the overall fold of the polypeptide chain is not disrupted. The energy-minimized structure showed good geometry, with bond lengths, bond angles, and main-chain and side-chain torsion angles were within the expected ranges of values. The overall self-compatibility score [78], as implemented in the Biosym/Homology system, which measures the compatibility of the sequence with the predicted three dimensional structure, was high and positive for the whole sequence. The link peptide that connects the N- and C-terminal CRD's did not have a counterpart in the template structures and could not be modeled. Secondary structure prediction programs (*e.g.* PHD [79]), suggested that this fragment is an irregular coil. The ribbon represents the predicted orientation of the polypeptide backbone of the N-CRD of galectin-8 (blue); C-CRD of galectin-8 (brown); and the known X-Ray structure of galectin-1 homodimer ([76] (purple)). The link peptide of galectin-8, whose predicted structure is unknown, is presented as an arbitrary random coil (white).

galectin-8, and other galectins as well, presumably can function outside the cells for prolonged periods of time in a non-reducing environment, without being inactivated.

Tissue distribution and subcellular localization of galectin-8

The expression of galectin-8 in different tissues was examined by Northern and Western blot analysis [1,17,18]. Unlike other galectins with a limited tissue distribution (*e.g.* galectin-4, [3]), galectin-8 is expressed in many tissues including lung, liver, kidney, spleen, hind-limb, and cardiac muscle. Low levels of expression were detected in intestine, colon, fat, and thymus. These results demonstrate that although galectin-8 is a fairly abundant protein, it is not ubiquitously expressed. Expression of galectin-8 is observed only in the cytoplasm. However, galectin-8 is not uniformly spread throughout the cytoplasm [1,14,17,25,27]. Instead, it shows a micro-clustering pattern reminiscent of that seen with proteins associated with mitochondria, the Golgi or trans-Golgi membranes. A similar

intracellular distribution was observed for some other galectins as well [28–30].

Expression of galectin-8 seems to be developmentally regulated. Very low levels of expression were noted in whole embryos [1], while high levels of expression were noted in adult tissues. In that respect galectin-8 might resemble other galectins that were implicated as regulators of cell growth [31,32], and embryogenesis [33].

Functional role of galectin-8

Galectin-8 as a mediator of cell adhesion

Extracellular matrix (ECM) proteins have an important function in providing structural integrity to tissues, and in presenting proper environmental cues for cell adhesion, migration, growth, and differentiation [34]. All of these aspects rely on the spatio-temporal expression of adhesive as well as anti-adhesive components in extracellular matrices and on the cell surface [35]. 'Classical' ECM proteins like fibronectin, collagen, and laminin were intensively studied, and their role as mediators of cell adhesion is best characterized [36]. Yet, cell adhesion also depends upon carbohydrate-protein interactions, mediated by mammalian lectins of different families [37]. Selectins function as mediators of cell-cell interactions [38], through calcium-dependent recognition of sialylated glycans [39]. Similarly, galectins were implicated as modulators of cell adhesion (reviewed in [40–42]) and were shown to enhance [20,43] or inhibit [19,44] cell-matrix interactions under different experimental setups.

Studies of galectin-8 revealed that galectin-8 positively or negatively regulates cell adhesion, depending on the extracellular context [18,45]. When immobilized onto matrix, galectin-8 can be classified as novel ECM protein equipotent to fibronectin in promoting cell adhesion, spreading and migration [45]. Accordingly, cell adhesion to galectin-8 triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK and paxillin. In contrast, excess soluble galectin-8 interacts both with cell surface integrins and with other soluble ECM proteins, such as fibronectin, and inhibits cell-matrix interactions.

Integrins mediate cell adhesion to galectin-8

Modulation of cell adhesion by galectin-8 is mediated upon interactions of galectin-8 with a selective subgroup of cell surface integrins. Several lines of evidence support this notion: First, soluble galectin-8 selectively inhibits cell adhesion to plates coated with integrin ligands laminin and fibronectin, while it fails to inhibit cell adhesion to the non-specific substratum polylysine [18,45]. More important, unlike fibronectin that binds most integrins [46], galectin-8 selectively interacts with a subgroup of integrin subunits that include α_3, α_6, and β_1 while it interacts to a very limited extent with α_4 and β_3 integrins [18]. Moreover, galectin-8 interactions with integrins involves its binding to sugar moieties, rather than the ligand-binding site

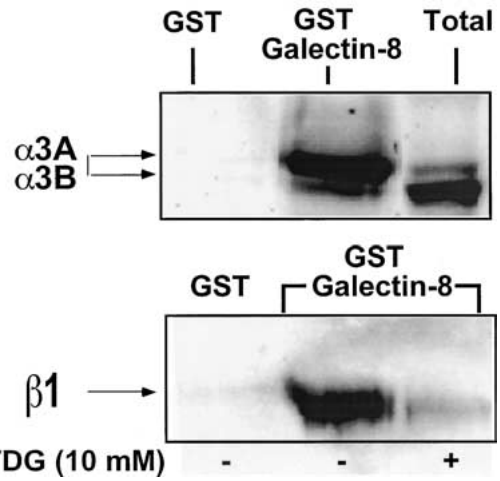


Figure 3. Binding of integrins to immobilized galectin-8. Cell Extracts of H1299 human lung carcinoma cells were incubated for 2 h at 4°C with immobilized-GST (2 mg) or with immobilized-GST-galectin-8 (1 mg) as described [18]. Whwn indicated, incubation was carried out in the presence of 10 mM TDG. Following incubation, the beads were washed and bound proteins were subjected to SDS-PAGE, transferred to a nitrocellulose membrane, blocked, and incubated with polyclonal anti-α3 integrin antibodies or (top) anti-β1 integrin antibodies (bottom).

on the extracellular domain of the integrin molecules. As shown in Figure 3, galectin-8 selectively interacts with sugar moieties of α_3A integrin, although this isoform is less abundant than its counterpart α_3B isoform. Hence, sugar residues at the extracellular domain of specific integrins like α_6, α_3A and β_1 are key cellular components which selectively bind galectin-8, making these integrins potential receptors that could mediate the effects of galectin-8 on cell adhesion. This idea is supported by the fact that β_1-integrin antibodies, directed towards protein epitopes, are less efficacious in inhibiting cell adhesion to immobilized galectin-8; that adhesion to galectin-8 is only partially sensitive to depletion of divalent cations (with EDTA), and it is completely insensitive to the presence of RGD peptides. Still, the ability of EDTA and Mn^{2+} to inhibit or potentiate, respectively, cell adhesion to galectin-8, suggests that the metal-bound conformation of integrins, is the preferred conformation that promotes adhesion to galectin-8.

Interaction of galectin-8 with integrins seems to be of physiological relevance. Immunofluorescence and immunohistochemical studies revealed that addition of rGalectin-8 to intact 1299 cells inhibited subsequent binding of α_3- or -β_1 antibodies, directed towards the extracellular region of these proteins [18], while inclusion of galectin-8 antibodies eliminated this inhibitory effect. These observations indicate that binding of galectin-8 to the cells masks essential antigenic determinants on the integrin surface, and supports the notion that galectin-8 interacts with integrins present in their native milieu within the context of an intact cell. Indeed, galectin-8 and $\alpha_3\beta_1$ form complexes in intact cells that can be isolated by precipitation with

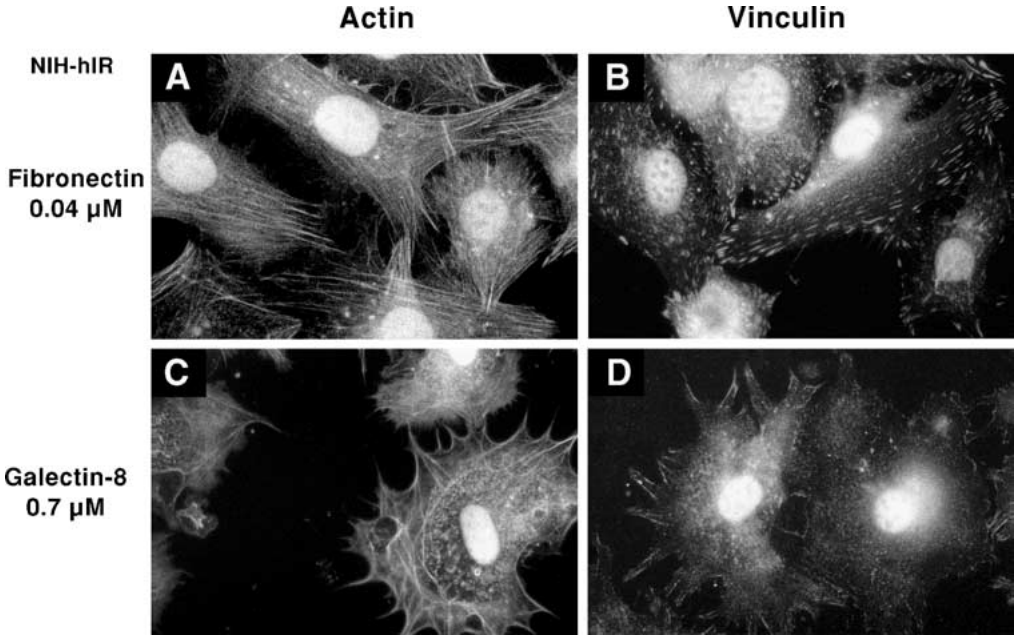


Figure 4. Cytoskeletal organization of cells adherent to galectin-8 or fibronectin. Cover glasses were precoated for 2 h at 22°C with 1 ml of fibronectin (0.04 μM) (A, B) or galectin-8 (0.7 μM) (C, D). NIH-hIR cells were grown on tissue culture plates and were incubated for 16 h in serum-free medium. Cells were detached from the culture plates with 5 mM EDTA, washed, and were incubated in suspension for 30 min at 37°C in serum-free medium. Cells were then seeded in serum-free medium on the coated cover glasses. Following 2 h incubation at 37°C cells were washed 3 times with PBS and fixed with paraformaldehyde (3%) containing 0.5% Triton X-100 for 5 min. Then, the cells were further incubated with paraformaldehyde (3%) for 30 min. Following 3 washes with PBS, cells were incubated for 1 h at 22°C with anti vinculin monoclonal antibody (B, D) or with TRITC-labeled phalloidine (A, C), all diluted in PBS. The specimens were washed 3 times with PBS and further incubated for 1 h at 22°C with secondary Cy3-conjugated goat anti-mouse antibodies in PBS (beside TRITC-labeled phalloidine specimens). Finally, the specimens were washed, mounted with elvanol onto glass microscope slides and examined on Zeiss fluorescence microscope.

specific α_3-antibodies [18]. These observations suggest that a secreted form of galectin-8 might act in an autocrine fashion when it remains bound to the extracellular regions of integrins expressed on the cell surface. Collectively, these findings implicate integrins as major cellular components through which a secreted galectin-8 manifests its biological activities.

Other galectins were also found to interact with integrins. Galectin-3 binds $\alpha_1\beta_1$ integrin [47]; galectin-1 interacts with integrin $\alpha_7\beta_1$ expressed on the surface of differentiating myoblasts [48], whereas $\alpha_M\beta_2$-integrin, otherwise known as CD11b/18 or complement receptor, is a major receptor for galectin-1 [49] and -3 [50]. Hence, different galectins might selectively regulate interactions of integrins with matrix proteins. The presence of a modified sugar-binding site at the N-CRD, and the unique sequences that flank this region [1,25], could confer upon galectin-8 its ability to interact with specific extracellular domains of integrins, not recognized by other galectins.

Cytoskeletal organization and signal cascades triggered upon cell adhesion onto galectin-8

Interaction of cell surface integrins with immobilized galectin-8 promotes cell attachment followed by cellular spreading. The ability of immobilized galectin-8 to promote both processes is evident from immunofluorescence studies which demonstrate the elaborated spreading of cells which takes place on immobilized galectin-8 (Figure 4). In fact, cells adhere and spread onto plates coated with galectin-8 with kinetics and efficacy similar to that found when cells adhere to fibronectin [45]. In spite of its functional similarity to other ECM proteins, galectin-8 manifests several distinct features. Most striking are the differences in cytoskeletal organization and focal contact formation induced by galectin-8, compared to those induced by fibronectin (Figure 4). While cells adherent onto fibronectin develop an elaborated network of actin bundles associated with well-developed focal contacts, cells attached to galectin-8 manifest a poorly organized network of actin microfilaments, with small focal contacts distributed mainly at the cell periphery. More importantly, many of these adhesion sites contain minimal amounts of vinculin or paxillin, which correlates with a reduced Tyr phosphorylation of paxillin [45], suggesting that other proteins presumably act in conjunction with integrins to propagate the intracellular signals evoked upon cell adhesion to galectin-8. This idea is not surprising in view of the fact that adhesion complexes show extraordinary structural and molecular diversity, and sites of cell adhesion to the ECM can be mediated by a variety of matrix molecules and integrin proteins [51].

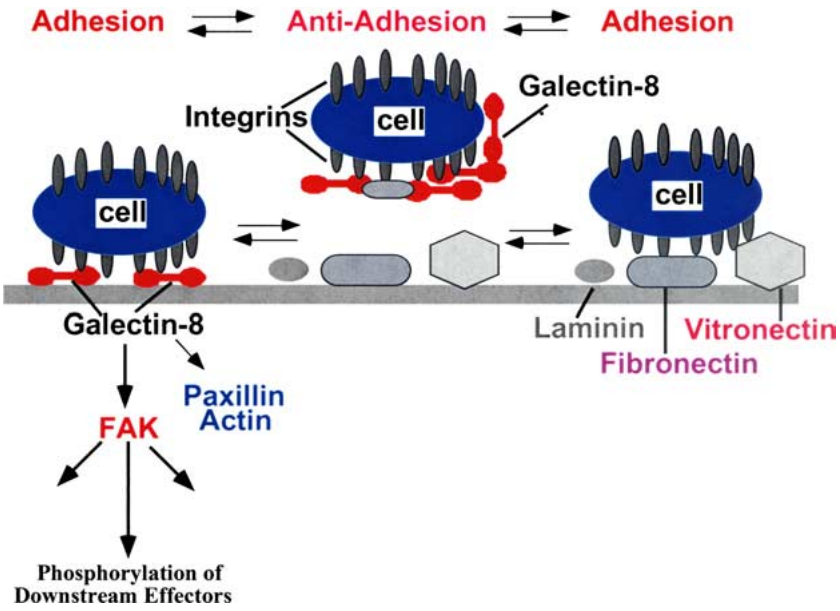
A related aspect is the Tyr phosphorylation of focal adhesion kinase (FAK), which takes place upon cell adhesion to galectin-8 [45]. This observation is consistent with the fact that galectin-8 by virtue of its two CRDs binds [18] and presumably aggregates β_1 integrins subunits, the main upstream activators of FAK [52]. Still, galectin-8-induced activation of FAK is independent of focal adhesion formation, a phenomena already observed in other cellular systems [53]. Hence, FAK phosphorylation which takes places during early stages of cell-matrix interactions is a common signal emitted upon cell adhesion to fibronectin or galectin-8. Thereafter, bifurcation of signals mediated by fibronectin and galectin-8 presumably takes place. Fibronectin recruits additional cytoskeletal elements like vinculin and paxillin, and induces their Tyr phosphorylation, while galectin-8 fails to do so and presumably emits a different set of signals [45]. Of relevance to this argument is the fact that galectin-8 effectively stimulates, better than fibronectin, the activities of protein kinase B, p70S6 kinase, as well as MAPK (Levy *et al*. unpublished) which is undetected in focal adhesions [52].

The differences in cytoskeletal organization between cells adherent to fibronectin vs. galectin-8 could be attributed to their different mode of interaction with integrins. Galectin-8 interactions with integrins involves its binding to sugar moieties, rather than the ligand-binding site on the extracellular domain of the integrin molecules. Hence, formation of protein-protein complexes upon binding of integrins to fibronectin, vs. the formation of protein-sugar complexes between galectin-8 and integrins offers a molecular aspect for the differences in cytoskeletal organization and signaling induced by these two matrices. Indeed, the less-developed pattern of actin filaments and focal contacts observed in cells seeded on galectin-8 resembles the appearance of cells whose integrins were aggregated in the absence of a ligand (*e.g*. RGD peptide) [54], suggesting that galectin-8, presumably fails to occupy the protein-ligand binding site of integrins, while it effectively induces aggregation of these receptors. The possibility that immobilized galectin-8 induces integrin clustering is consistent with the fact that a truncated form of galectin-8, which contains only its N-terminal half with a single CRD, is much less efficient (about 5 fold) in functioning as an ECM protein that promotes cell adhesion [45]. These findings suggest that ligation of cell surface integrins is necessary but insufficient to trigger the biological functions of immobilized galectin-8, and receptor clustering, in addition to receptor occupancy, is required to promote the adhesive effects of galectin-8. In that respect, galectin-8 resembles "classical" ECM proteins that induce integrin aggregation to trigger cell adhesion and to initiate the signaling cascaded generated thereof [55,56].

Cell adhesion to immobilized galectin-8 is inhibited in the presence of serum. These findings suggest that complex formation between galectin-8 and serum components generates a matrix that attenuates cell adhesion. This phenomenon is compatible with the ability of galectin-8 to promote cell motility. Indeed, cells readily migrate on plates coated with galectin-8 (in the presence of serum) indicating that complexes of galectin-8 with matrix/serum components are presumably advantageous when cellular migration is in effect and their formation might play a physiological role in the regulation of cellular motility. Galectin-8 binds serum fibronectin [45] by virtue of its affinity for glycans carrying multiple *N*-acetyllactosamine units. Other galectins were also shown to bind to certain isoforms of laminin and fibronectin that express such glycans [41,44]. Still, generation of fibronectin-galectin-8 complexes does not seem to interfere with cell adhesion since cell adhesion to immobilized galectin-8 is not interrupted in the presence of purified fibronectin [45].

Cell adhesion is also inhibited by exogenously-added soluble galectin-8. In that respect galectin-8 resembles other soluble ECM proteins like laminin [57] and fibronectin [58,59] that inhibit cell adhesion upon binding to integrins. Hence, perturbation of the physiological galectin-integrin complexes, upon addition of excess soluble galectin-8, presumably masks the integrin ligand binding sites and thus impairs cell adhesion to integrin ligands such as fibronectin. Alternatively, soluble galectin-8 could induce the internalization of cell surface integrins and in such a way impair cell adhesion. This possibility is supported by recent findings indicating that galectin-3 can mediate the endocytosis of $\beta 1$ integrins [60]. The effects of soluble galectin-8 resemble other galectins, such as galectin-1 and galectin-3 that can induce internalization of cell adhesion receptors, or can induce steric hindrance when bound monovalently to cellular receptors or to matrix proteins (cf. [41] for a recent review). Inhibition of cell adhesion induced by soluble galectin-8 is further complicated by the fact that galectin-8 also binds soluble fibronectin present in the serum [18]. Hence, the anti-adhesive effects of galectin-8 could be mediated either upon direct binding of excess soluble galectin-8 to cell surface integrins, or alternatively, upon binding and recruitment to the cell surface of other soluble ECM proteins such as fibronectin that, when soluble, could exert an anti-adhesive effect of their own [58,59]. This function requires the occupancy of both CRDs of the native galectin-8, and might account for the inability of the truncated monovalent soluble N-galectin-8 to inhibit cell adhesion. The inhibitory effects of galectin-8 were reversed by manganese ions, a powerful activator of integrins, suggesting that ligation by soluble galectin-8 may stabilize integrins in a low affinity state [18]. Altogether, the function of soluble galectin-8 seems to be dictated by the combinatorial arrangement of available cell surface and extracellular ligands (Scheme 1).

Due to its pro- and anti-adhesive functions galectin-8 can be considered as a novel member of adhesion-modulating proteins such as SPARC, thrombospondin, tenascin, hevin and disintegrins [61–63], collectively known as matricellular proteins. Still, it should be emphasized that the action of matricellular proteins depends upon protein-protein interactions, while galectin-8's function depends upon sugar-protein interactions.



Scheme 1. Function of Galectin-8 as a matricellular protein. Immobilized galectin-8 functions as a matrix protein equipotent to fibronectin in promoting cell adhesion by ligation and clustering sugar moieties of cell surface integrin receptors. Adhesion to galectin-8 triggers integrin-mediated signaling cascades such as Tyr phosphorylation of FAK and paxillin. In contrast, when present in excess as a soluble ligand, galectin-8 interacts both with integrins and other soluble matrix proteins such as fibronectin, to negatively regulate cell-matrix interactions in a sugar-dependent manner. Such mechanism allows local signals emitted by galectin-8 to specify territories non accessible for cell adhesion. This implicates members of the galectin family as a novel class of matricellular proteins. Taken from [45].

Such usage of the glyco-code adds a novel biological role for lectin-carbohydrate interactions.

Galectin-8 as a mediator of cell growth

The inhibitory effects of soluble galectin-8 on cell adhesion correspond to its effects on cellular growth. In line with this idea are the experiments demonstrating inhibition of colony formation in lung carcinoma cells transfected with the gene coding for galectin-8 [18]. A similar effect was observed, when another tandem-repeat type, galectin-12, was overexpressed in HeLa cells [6]. The inhibitory effects of the overexpressed galectin-8 could be accounted for by an autocrine effect of the secreted lectin that interacts with the available cell surface integrins, thus preventing altogether cell adhesion, similar to its anti inhibitory role when added as a soluble ligand. Alternatively, galectin-8 could act intracellularly and inhibit cell growth by as yet undefined mechanism.

Inhibition of matrix-integrins interactions has been implicated in the induction of apoptosis in cells denied of anchorage [64,65]. Indeed, galectin-8 is an effective inducer of an apoptotic process in several cell types [18]. Apoptosis, like binding to integrins, is prevented upon inclusion of thiodigalactoside, suggesting that both events are at least partially mediated by interactions of galectin-8 with cell surface glycoconjugates. However, apoptosis induced by galectin-8 is not secondary to its anti adhesive signals, because it takes place when galectin-8 is applied to adherent cells that do not detach during the course of the experiment. These findings suggest that soluble galectin-8 might induce its anti adhesive and apoptotic effects by two independent mechanisms. Furthermore, serum constituents might act as survival factors to rescue the cells from apoptotic signals transmitted by galectin-8. Of note, apoptosis induced by galectin-8 differs from that induced by galectin-1 [66], which is serum-independent, again suggesting that these two lectins might act through different mechanisms.

Galectin-8 and cancer

In recent years extensive investigation took place in order to unmask the complicated role of galectins in many aspects of human neoplasms (cf. [67–69]). Being only recently examined in this context, galectin-8 was shown to have different tumor modulating properties in various human neoplasms, both in *in-vitro* and *in-vivo* (using human tumor xenografts) experiments. Secretion of galectin-8 is markedly enhanced, and its surface expression is highly increased in invasive human prostate carcinoma, early prostate cancer and prostatic intraepithelial neoplasia, but not on normal prostate or benign prostatic hypertrophy tissues [14]. For this reason it was formerly called PCTA-1 (Prostate Cancer Tumor Antigen-1) [14]. High levels of galectin-8 expression is also observed in human lung carcinoma cells. Of the group of other tumors in which high galectin-8 levels were detected are central nervous system astrocytoma and glioblastoma, the cutaneous T cell Lymphoma,

and the middle ear cholesteatoma [70–72]. Galectin-8 mRNA was the most abundantly expressed message of galectins, whose presence was monitored in 61 human tumor cell lines of different origin (breast, colon, lung, brain, skin, kidney, urogenital system, and hematopoietic system). Expression of galectin-8 was found in all but two of the tested cells [73]. Furthermore, galectin-8 localizes to the invasive parts of certain glioblastomas explanted into brains of nude mice [70]. Hence, overexpression of galectin-8 may represent a key attribute associated with progression of certain types of tumors. On the other hand, in Colon carcinoma, the level of galectin-8 expression was found to decrease markedly during tumorigenesis [74]. Conflicting evidences came from the benign metastasizing uterine leiomyoma, where galectin-8 was detected in the minority of cases studied [75]. Altogether, it seems reasonable to postulate that galectin-8 levels of expression are not ubiquitous in all human neoplasms, but are closely correlated with specific neoplasms, providing them with some growth and metastasis related advantages.

Currently, there are at least two approaches under investigation concerning the potential role of galectin-8 in prostate cancer research and treatment. The first is the possibility it may increase the sensitivity and specificity or even replace the traditional prostate serum antigen (PSA) screening by permitting a clear distinction between prostate carcinoma *versus* normal gland and BPH, due to the fact it was not found to be expressed neither by normal prostate tissue, nor by hyperplastic prostate tissue [14]. The second approach pursues the idea that galectin-8, due to its modulatory effects on cell adhesion, may even serve to modify the tumor biology and natural history of the tumor growth.

In summary, interactions of a secreted galectin-8 with cell surface integrins inhibits cell adhesion, while immobilized galectin-8 has the potential to promote cellular attachment, spreading and migration, therefore galectin-8 may modulate cell-matrix interactions in a variety of physiological and pathological processes.

Future perspectives

Since the discovery of galectin-8, certain progress has been made in attempts to unravel its structure, localization, and some of its physiological functions. Like other galectins, galectin-8 is expected to be a secreted ligand that plays a central role in fundamental cellular processes such as cell adhesion, cell growth and apoptosis. Still we are far beyond achieving a clear understanding of the biological role of galectin-8. Different experimental approaches are likely to advance our knowledge. Better understanding of the signaling cascades emitted upon cell adhesion to galectin-8, combined with analysis of the mechanisms involved in its effects on cell cycle progression could help clarify the mode of action of this lectin. Similarly, detailed analysis of the sugar-binding specificity of galectin-8 and identification of galectin-8-binding proteins are likely to produce useful information. Generation of transgenic mice that overexpress galectin-8, and production of mice that lack the galectin-8 genes are obvious avenues that should be avidly pursued. Ligation of integrins by ECM proteins triggers transcription of a unique set of genes. Accordingly, identification of genes whose expression is modulated upon cell adhesion to galectin-8 may help in elucidating its biological activity. Collectively, these examples of possible experimental approaches, are likely to converge into a major undertaking aimed to enlighten novel aspects related to the mode of action of galectin-8 under physiological and pathological conditions.

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References

1 Hadari YR, Paz K, Dekel R, Mestrovic T, Accili D, Zick Y, Galectin-8. A new rat lectin, related to galectin-4, *J Biol Chem* **270**, 3447–53 (1995).
2 Hirabayashi J, Satoh M, Kasai K, Evidence that Caenorhabditis elegans 32-kDa beta-galactoside-binding protein is homologous to vertebrate beta-galactoside-binding lectins. cDNA cloning and deduced amino acid sequence, *J Biol Chem* **267**, 15485–90 (1992).
3 Oda Y, Herrmann J, Gitt MA, Turck CW, Burlingame AL, Barondes SH, Leffler H, Soluble lactose-binding lectin from rat intestine with two different carbohydrate-binding domains in the same peptide chain, *J Biol Chem* **268**, 5929–39 (1993).
4 Gitt MA, Colmot C, Xia YR, Atchison RE, Lusis AJ, Poirier F, Barondes S, Leffler H, Galectin-6: A new mammalian Galectin, *Glycoconjugate J* **12**, 548 (1996).
5 Wada J, Kanwar YS, Identification and characterization of galectin-9, a novel beta-galactoside-binding mammalian lectin, *J Biol Chem* **272**, 6078–86 (1997).
6 Yang RY, Hsu DK, Yu L, Ni J, Liu FT, Cell cycle regulation by galectin-12, a new member of the galectin superfamily, *J Biol Chem* **276**, 20252–60 (2001).
7 Wang JL, Laing JG, Anderson RL, Lectins in the cell nucleus, *Glycobiology* **1**, 243–52 (1991).
8 Drickamer K, Taylor ME, Biology of animal lectins, *Annu Rev Cell Biol* **9**, 237–64 (1993).
9 Rini JM, Lobsanov YD, New animal lectin structures, *Curr Opin Struct Biol* **9**, 578–84 (1999).
10 Leffler H, Galectins structure and function—A synopsis, *Results Probl Cell Differ* **33**, 57–83 (2001).
11 Liao F, Shin HS, Rhee SG, Tyrosine phosphorylation of phospholipase C-gamma 1 induced by cross- linking of the high-affinity or low-affinity Fc receptor for IgG in U937 cells, *Proc Natl Acad Sci USA* **89**, 3659–63 (1992).
12 Lobsanov YD, Gitt MA, Leffler H, Barondes SH, Rini JM, X-ray crystal structure of the human dimeric S-Lac lectin, L-14-II, in complex with lactose at 2.9-A resolution, *J Biol Chem* **268**, 27034–38 (1993).

13 Hirabayashi J, Kasai K, Effect of amino acid substitution by sited-directed mutagenesis on the carbohydrate recognition and stability of human 14-kDa beta-galactoside- binding lectin, *J Biol Chem* **266**, 23648–53 (1991).
14 Su Z-Z, Lin J, Shen R, Fisher PE, Goldstein NI, Fisher PB, Surface-epitope masking and expression cloning identifies the human prostate carcinoma tumor antigen PCTA-1 a member of the galectin gene family, *Proc Natl Acad Sci USA* **93**, 7252–7 (1996).
15 Bidon N, Brichory F, Hanash S, Bourguet P, Dazord L, Le PJ, Two messenger RNAs and five isoforms for Po66-CBP, a galectin-8 homolog in a human lung carcinoma cell line, *Gene* **274**, 253–62 (2001).
16 Bidon N, Brichory F, Bourguet P, Le PJ, Dazord L, Galectin-8: A complex sub-family of galectins (Review), *Int J Mol Med* **8**, 245–50 (2001).
17 Gopalkrishnan RV, Roberts T, Tuli S, Kang D, Christiansen KA, Fisher PB, Molecular characterization of prostate carcinoma tumor antigen-1, PCTA-1, a human galectin-8 related gene, *Oncogene* **19**, 4405–16 (2000).
18 Hadari YR, Goren R, Levy Y, Amsterdam A, Alon R, Zakut R, Zick Y, Galectin-8 binding to integrins inhibits cell adhesion and induces apoptosis, *J Cell Sci* **113**, 2385–97 (2000).
19 Cooper DN, Massa SM, Barondes SH, Endogenous muscle lectin inhibits myoblast adhesion to laminin, *J Cell Biol* **115**, 1437–48 (1991).
20 Kuwabara I, Liu FT, Galectin-3 promotes adhesion of human neutrophils to laminin, *J Immunol* **156**, 3939–44 (1996).
21 Cleves AE, Cooper DN, Barondes SH, Kelly RB, A new pathway for protein export in Saccharomyces cerevisiae, *J Cell Biol* **133**, 1017–26 (1996).
22 Rubartelli A, Bajetto A, Allavena G, Wollman E, Sitia R, Secretion of thioredoxin by normal and neoplastic cells through a leaderless secretory pathway, *J Biol Chem* **267**, 24161–4 (1992).
23 Siders WM, Klimovitz JC, Mizel SB, Characterization of the structural requirements and cell type specificity of IL-1 alpha and IL-1 beta secretion, *J Biol Chem* **268**, 22170–74 (1993).
24 Mignatti P, Rifkin DB, Release of basic fibroblast growth factor, an angiogenic factor devoid of secretory signal sequence: A trivial phenomenon or a novel secretion mechanism? *J Cell Biochem* **47**, 201–7 (1992).
25 Hadari YR, Eisenstein M, Zakut R, Zick Y, Galectin-8: On the road from structure to function, *TIGG* **9**, 103–12 (1997).
26 Kasai K-I, Hirabayashi J, Galectins: A family of animal lectins that decipher glycocodes, *J Biochem* **119**, 1–8 (1996).
27 Bassen R, Brichory F, Caulet-Maugendre S, Bidon N, Delaval P, Desrues B, Dazord L, Expression of Po66-CBP, a type-8 galectin, in different healthy, tumoral and peritumoral tissues, *Anticancer* **19**, 5429–33 (1999).
28 Hadj SY, Seve AP, Doyennette MM, Saffar L, Felin M, Aubery M, Gattegno L, Hubert J, Nuclear and cytoplasmic expressions of the carbohydrate-binding protein CBP70 in tumoral or healthy cells of the macrophagic lineage, *J Cell Biochem* **62**, 529–42 (1996).
29 Sarafian V, Jadot M, Foidart JM, Letesson JJ, Van-den-Brule F, Castronovo V, Wattiaux R, Coninck SW, Expression of Lamp-1 and Lamp-2 and their interactions with galectin-3 in human tumor cells, *Int J Cancer* **75**, 105–11 (1998).
30 Maldonado CA, Castagna LF, Rabinovich GA, Landa CA, Immunocytochemical study of the distribution of a 16-kDa galectin in the chicken retina, *Invest Ophthalmol Vis Sci* **40**, 2971–17 (1999).
31 Sanford GL, Harris HS, Stimulation of vascular cell proliferation by beta-galactoside specific lectins, *FASEB J* **4**, 2912–8 (1990).
32 Wells V, Mallucci L, Identification of an autocrine negative growth factor: Mouse beta-galactoside binding protein is a cytostatic factor and cell growth regulator, *Cell* **64**, 91–7 (1991).
33 Poirier F, Timmons PM, Chan CT, Guenet JL, Rigby PW, Expression of the L14 lectin during mouse embryogenesis suggests multiple roles during pre- and post-implantation development, *Development* **115**, 143–55 (1992).
34 Retta SF, Ternullo M, Tarone G, Adhesion to matrix proteins, *Methods Mol Biol* **96**, 125–30 (1999).
35 Chiquet-Ehrismann R, Inhibition of cell adhesion by anti-adhesive molecules, *Curr Op Cell Biol* **7**, 715–9 (1995).
36 Geiger B, Bershadsky A, Pankov R, Yamada KM, Transmembrane extracellular matrix-cytoskeleton crosstalk, *Nat Rev Mol Cell Biol* **2**, 793–805 (2001).
37 Sharon N, Lis H, Lectins-proteins with a sweet tooth: Function in cell recognition, *Essays in Biochemistry* **30**, 59–75 (1995).
38 Springer TA, Traffic signals on endothelium for lymphocyte recirculation and leukocyte emigration, *Annu Rev Physiol* **57**, 827–72 (1995).
39 Vestweber D, Blanks JE, Mechanisms that regulate the function of the selectins and their ligands, *Physiol Rev* **79**, 181–213 (1999)
40 Kaltner H, Stierstorfer B, Animal lectins as cell adhesion molecules, *Acta Anat (Basel)* **161**, 162–79 (1998).
41 Hughes RC, Galecins as modulators of cell adhesion, *Biochimie* **83**, 667–76 (2001).
42 Rabinovich GA, Baum LG, Tinari N, Paganelli R, Natoli C, Liu FT, Iacobelli S, Galectins and their ligands: Amplifiers, silencers or tuners of the inflammatory response? *Trends Immunol* **23**, 313–20 (2002).
43 Mahanthappa NK, Cooper DNW, Barondes SH, Schwarting GA, Rat olfactory neurins can utilize the endogenous lectin, L-14, in a novel adhesion mechanism, *Development* **120**, 1373–84 (1994).
44 Sato S, Hughes RC, Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin, *J Biol Chem* **267**, 6983–90 (1992).
45 Levy Y, Arbel-Goren R, Hadari YR, Ronen D, Bar-Peled O, Elhanany E, Geiger B, Zick Y, Galectin-8 functions as a matricellular modulator of cell adhesion, *J Biol Chem* **276**, 31285–95 (2001).
46 Schwarzbauer JE, Sechler JL, Fibronectin fibrillogenesis: A paradigm for extracellular matrix assembly, *Curr Opin Cell Biol* **11**, 622–7 (1999).
47 Ochieng J, Leite BM, Warfield P, Regulation of cellular adhesion to extracellular matrix proteins by galectin-3, *Biochem Biophys Res Commun* **246**, 788–91 (1998).
48 Gu M, Wang W, Song WK, Cooper DN, Kaufman SJ, Selective modulation of the interaction of alpha 7 beta 1 integrin with fibronectin and laminin by L-14 lectin during skeletal muscle differentiation, *J Cell Sci* **107**, 175–81 (1994).
49 Avni O, Pur Z, Yefenof E, Baniyash M, Complement receptor 3 of macrophages is associated with galectin-1-like protein, *J Immunol* **160**, 6151–8 (1998).
50 Dong S, Hughes RC, Macrophage surface glycoproteins binding to galectin-3 (Mac-2-antigen), *Glycoconj J* **14**, 267–74 (1997).
51 Zamir E, Katz M, Posen Y, Erez N, Yamada KM, Katz BZ, Lin S, Lin DC, Bershadsky A, Kam Z, Geiger B, Dynamics and

segregation of cell-matrix adhesions in cultured fibroblasts, *Nat Cell Biol* **2**, 191–6 (2000).
52 Yamada KM, Miyamoto S, Integrin transmembrane signaling and cytoskeletal control, *Curr Opin Cell Biol* **7**, 681–9 (1995).
53 Lyman S, Gilmore A, Burridge K, Gidwitz S, White II GC, Integrin-mediated activation of focal adhesion kinase is independent of focal adhesion formation or integrin activation. Studies with activated and inhibitory beta3 cytoplasmic domain mutants, *J Biol Chem* **272**, 22538–47 (1997).
54 Miyamoto S, Teramoto H, Coso OA, Gutkind JS, Burbelo PD, Akiyama SK, Yamada KM, Integrin function: Molecular hierarchies of cytoskeletal and signaling molecules, *J Cell Biol* **131**, 791–805 (1995).
55 Hynes RO, Cell adhesion: Old and new questions, *Trends Cell Biol* **9**, M33–7 (1999)
56 Giancotti FG, Ruoslahti E, Integrin signaling, *Science* **285**, 1028–32 (1999).
57 Calof AL, Campanero MR, O'Rear JJ, Yurchenco PD, Lander AD, Domain-specific activation of neuronal migration and neurite outgrowth-promoting activities of laminin, *Neuron* **13**, 117–30 (1994).
58 Yamada KM, Kennedy DW, Dualistic nature of adhesive protein function: Fibronectin and its biologically active peptide fragments can autoinhibit fibronectin function, *J Cell Biol* **99**, 29–36 (1984).
59 Woods ML, Cabanas C, Shimizu Y, Activation-dependent changes in soluble fibronectin binding and expression of beta1 integrin activation epitopes in T cells: Relationship to T cell adhesion and migration, *Eur J Immunol* **30**, 38–49 (2000).
60 Furtak V, Hatcher F, Ochieng J, Galectin-3 mediates the endocytosis of beta-1 integrins by breast carcinoma cells, *Biochem Biophys Res Commun* **289**, 845–50 (2001).
61 Murphy UJ, Lightner VA, Aukhil I, Yan YZ, Erickson HP, Hook M, Focal adhesion integrity is downregulated by the alternatively spliced domain of human tenascin, *J Cell Biol* **115**, 1127–36 (1991).
62 Sage EH, Bornstein P, Extracellular proteins that modulate cell-matrix interactions. SPARC, tenascin, thrombospondin, *J Biol Chem* **266**, 14831–4 (1991).
63 Girard JP, Springer TA, Modulation of endothelial cell adhesion by hevin, an acidic protein associated with high endothelial venules, *J Biol Chem* **271**, 4511–7 (1996).
64 Ruoslahti E, Reed CJ, Anchorage dependence, integrins, and apoptosis, *Cell* **77**, 477–8 (1994).
65 Frisch SM, Francis H, Disruption of epithelial cell-matrix interactions induces apoptosis, *J Cell Biol* **124**, 619–26 (1994).
66 Perillo NL, Pace KE, Seilhamer JJ, Baum LG, Apoptosis of T cells mediated by galectin-1, *Nature* **378**, 736–9 (1995).
67 Gorelik E, Galili U, Raz A, On the role of cell surface carbohydrates and their binding proteins (lectins) in tumor metastasis, *Cancer Metastasis Rev* **20**, 245–77 (2001).
68 Camby I, Belot N, Lefranc F, Sadeghi N, de LY, Kaltner H, Musette S, Darro F, Danguy A, Salmon I, Gabius HJ, Kiss R, Galectin-1 modulates human glioblastoma cell migration into the brain through modifications to the actin cytoskeleton and levels of expression of small GTPases, *J Neuropathol Exp Neurol* **61**, 585–96 (2002).
69 Deininger MH, Trautmann K, Meyermann R, Schluesener HJ, Galectin-3 labeling correlates positively in tumor cells and negatively in endothelial cells with malignancy and poor prognosis in oligodendroglioma patients, *Anticancer Res* **22**, 1585–92 (2002).
70 Camby I, Belot N, Rorive S, Lefranc F, Maurage CA, Lahm H, Kaltner H, Hadari Y, Ruchoux MM, Brotchi J, Zick Y, Salmon I, Gabius HJ, Kiss R, Galectins are differentially expressed in supratentorial pilocytic astrocytomas, astrocytomas, anaplastic astrocytomas and glioblastomas, and significantly modulate tumor astrocyte migration, *Brain Pathol* **11**, 12–26 (2001).
71 Wollina U, Graefe T, Feldrappe S, Andre S, Wasano K, Kaltner H, Zick Y, Gabius HJ, Galectin fingerprinting by immuno- and lectin histochemistry in cutaneous lymphoma, *J Cancer Res Clin Oncol* **128**, 103–10 (2002).
72 Simon P, Decaestecker C, Choufani G, Delbrouck C, Danguy A, Salmon I, Zick Y, Kaltner H, Hassid S, Gabius HJ, Kiss R, Darro F. The levels of retinoid RARbeta receptors correlate with galectin-1, -3 and -8 expression in human cholesteatomas, *Hear Res* **156**, 1–9 (2001).
73 Lahm H, Andre S, Hoeflich A, Fischer JR, Sordat B, Kaltner H, Wolf E, Gabius HJ, Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures, *J Cancer Res Clin Oncol* **127**, 375–86 (2001).
74 Nagy N, Bronckart Y, Camby I, Legendre H, Lahm H, Kaltner H, Hadari Y, Van HP, Yeaton P, Pector JC, Zick Y, Salmon I, Danguy A, Kiss R, Gabius HJ, Galectin-8 expression decreases in cancer compared with normal and dysplastic human colon tissue and acts significantly on human colon cancer cell migration as a suppressor, *Gut* **50**, 392–401 (2002).
75 Kayser K, Zink S, Schneider T, Dienemann H, Andre S, Kaltner H, Schuring MP, Zick Y, Gabius HJ, Benign metastasizing leiomyoma of the uterus: Documentation of clinical, immunohistochemical and lectin-histochemical data of ten cases, *Virchows Arch* **437**, 284–92 (2000).
76 Liao DI, Kapadia G, Ahmed H, Vasta GR, Herzberg O, Structure of S-lectin, a developmentally regulated vertebrate beta- galactoside-binding protein, *Proc Natl Acad Sci USA* **91**, 1428–32 (1994).
77 Levitt M, Molecular dynamics of native protein, I: Computer simulation of trajectories, *J Mol Biol* **168**, 595–620 (1983).
78 Bowie JU, Luthy R, Eisenberg D, A method to identify protein sequences that fold into a known three-dimensional structure, *Science* **253**, 164–70 (1991).
79 Rost B, Sander C, Combining evolutionary information and neural networks to predict protein secondary structure, *Proteins* **19**, 55–72 (1994).